

Amendments to the Claims:

The following listing of claims will replace all prior versions and listings of claims in this application:

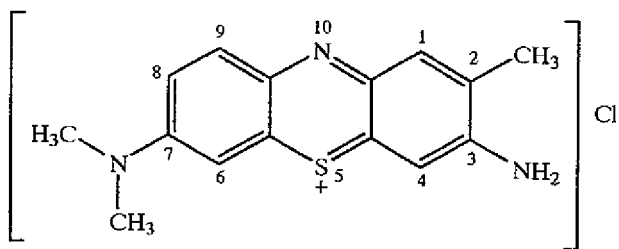
What is claimed:

1. (CANCELLED).
2. (CANCELLED).
3. (CANCELLED).
4. (CANCELLED).
5. (CANCELLED).

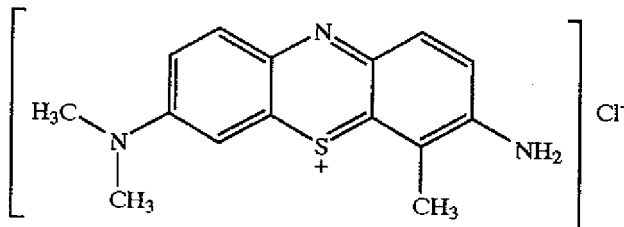
6. (NEW) A prognostic method for early prediction of eventual development of invasive epithelial cancer, said method comprising:

(a) applying to epithelial tissue having DNA a staining dye that stains neoplastic and preneoplastic cells, wherein the dye comprises:

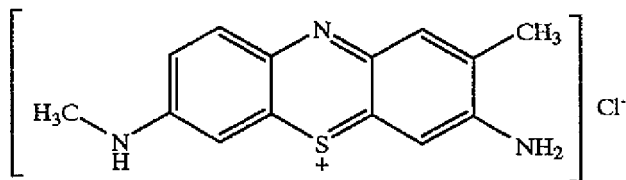
(1) the conformational isomers of toluidine blue O, the compounds having the structures



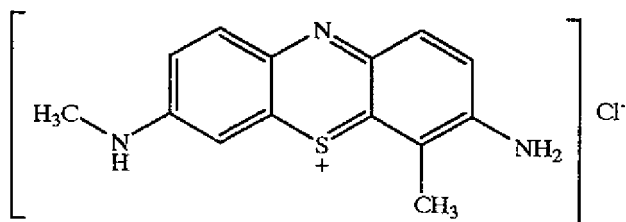
and



(2) the N-demethylation derivatives of said isomers, the compounds having the structures



and



the ratio of the combined areas of the 254 nm HPLC peaks representing said isomers to the combined areas of the peaks representing said N-demethylation derivatives being at least about 6:1;

(b) identifying clonal patches of said epithelial tissue by visually examining said tissue for stained tissue sites;

(c) resecting epithelial tissue having said DNA in the locus of said clonal patches;

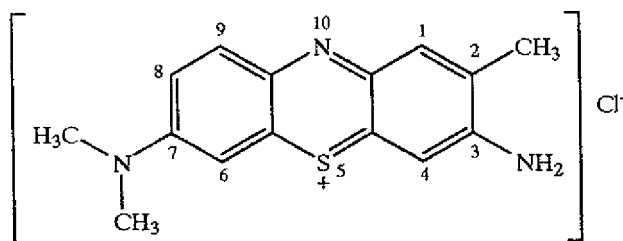
(d) extracting said DNA from said resected epithelial tissue; and

(e) making said prognosis by determining whether DNA extracted from said resected epithelial tissue exhibits allelic losses or mutations of tumor suppressor genes, which losses or mutation are indicative that said resected tissue is in the progression pathway to development of invasive cancer.

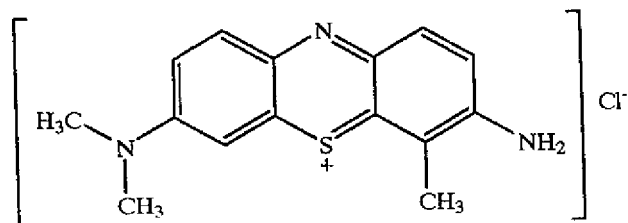
7. (NEW) A method for determining the likelihood of cancer in epithelial tissue, the method comprising:

(a) applying a dye that stains neoplastic and preneoplastic cells to the epithelial tissue, wherein the dye comprises :

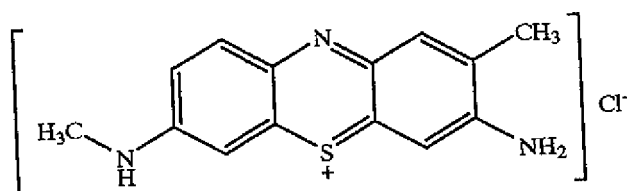
(1) the conformational isomers of toluidine blue O, the compounds having the structures



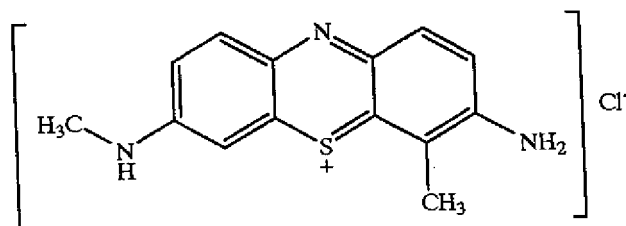
and



(2) the N-demethylation derivatives of said isomers, the compounds having the structures



and



the ratio of the combined areas of the 254 nm HPLC peaks representing said isomers to the combined areas of the peaks representing said N-demethylation derivatives being at least about 6:1;

(b) subjecting the epithelial tissue that is stained with the dye to microsatellite analysis to determine whether the DNA from the stained epithelial

tissue exhibits loss of heterozygosity (LOH) of at least one chromosome;
wherein the LOH of at least one chromosome indicates a risk of future progression to epithelial cancer.

8. (NEW) The method of Claim 7, wherein the microsatellite analysis is conducted at any one or a combination of the following chromosomes: 3p, 9p, and 17p.

9. (NEW) The method of claim 8, wherein LOH of 3p and/or 9p indicates a 28-75% likelihood of the future progression to epithelial cancer.

10. (NEW) The method of Claim 8, wherein the LOH of 3p, 9p, and 17p indicates a high likelihood of the future progression of epithelial cancer.

11. (NEW) The method of claim 6, wherein the dye is retained in the mitochondria of neoplastic and preneoplastic cells.

12. (NEW) The method of claim 7, wherein the dye is retained in the mitochondria of the neoplastic and preneoplastic cells.

13. (NEW) A prognostic method for early prediction of eventual development of invasive cancer, said method comprising:

(a) applying to epithelial tissue having DNA a staining dye that stains neoplastic and preneoplastic cells, wherein the dye is made using a process for

manufacturing toluidine blue O, which comprises the steps of

(1) oxidizing N,N-dimethyl-p-phenylene diamine in a first reaction mixture, to form a first intermediate, 2-amino-5-dimethylaminophenyl thiosulfonic acid,

(2) oxidizing said first intermediate and condensing the oxidizate in a second reaction mixture with o-toluidine, forming a second intermediate, indamine thiosulfonic acid,

(3) oxidizing said second intermediate to close the indamine ring thereof, forming a TBO-containing reaction product dissolved in a third reaction mixture,

(4) introducing a complexing reagent into said third reaction mixture, to form a TBO-complex product dissolved in said third reaction mixture,

(5) precipitating said TBO-complex product from said third reaction mixture, and

(6) separating said TBO-complex product, containing the conformation isomers of TBO;

wherein the improved process comprises introducing said complexing reagent to a reaction mixture before the formation of said third reaction mixture, said complexing reagent being a compound that forms with said N,N-dimethyl-p-phenylenediamine said first intermediate

and/or said second intermediate, a complex that provides steric hinderence to demethylation thereof;

(b) identifying clonal patches of said epithelial tissue by visually examining said tissue for stained tissue sites;

(c) resecting epithelial tissue having said DNA in the locus of said clonal patches;

(d) extracting said DNA from said resected epithelial tissue; and

(e) making said prognosis by determining whether DNA extracted from said resected epithelial tissue exhibits allelic losses or mutations of tumor suppressor genes, which losses or mutation are indicative that said resected tissue is in the progression pathway to development of invasive cancer.

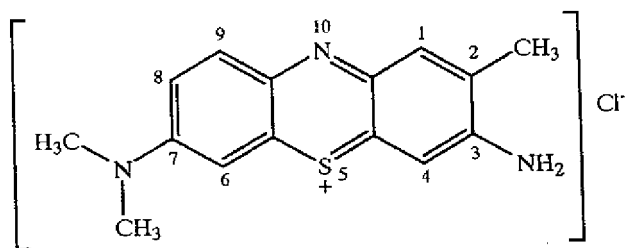
14. (NEW) The method of claim 13, wherein the dye stains mitochondria of neoplastic and preneoplastic cells.

15. (NEW) A prognostic method for the early prediction of eventual development of invasive epithelial cancer, said method comprising:

(a) applying to epithelial tissue having DNA a staining dye that stains neoplastic and preneoplastic cells, wherein the dye comprises:

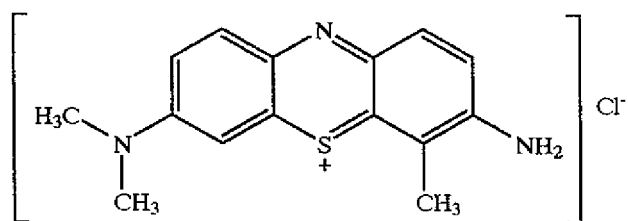
the conformational isomers of toluidine blue O, the compounds having the structures

(i)



and

(ii)



in which (i) comprises at least 58% of the total organic dye content of said composition.